

Post-Embryonic Development of the Horseshoe Crab¹

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Abstract. Individuals of *Limulus polyphemus* and *Tachypleus tridentatus* reached, respectively, the fourteenth-instar and tenth-instar stages during our nine-year rearing experiment. Body sizes were measured using exuviae and body specimens. The results made their growth steps clear, and body sizes of the juveniles at further growth stages could be estimated. We conclude from these data that *L. polyphemus* males generally molt 16 times to reach maturity in the ninth year; females molt 17 times to reach maturity in the tenth year. Similarly, we conclude from the presumptive numbers of growth stages that *T. tridentatus* males generally molt 15 times to reach maturity in the thirteenth year; females molt 16 times to reach maturity in the fourteenth year. Although we have few data on growth stages of *Tachypleus gigas* and *Carcinoscorpius rotundicauda*, it is thought that *T. gigas* males molt 12 times to reach adulthood, females molt 13 times, and that *C. rotundicauda* reaches maturity after the thirteenth molt in both males and females.

Introduction

Few have studied the post-embryonic development of horseshoe crabs. This may be because a method of culturing juveniles had not been established and that, in *Ta-*

chypleus tridentatus, first instars did not grow into second instars within a year of hatching. Recently, Brown and Clapper (1981) described the procedures for collecting gametes, culturing embryos and juveniles, and maintaining adults of *Limulus polyphemus*. Until that time, they grew *Limulus* juveniles to the fifth-instar stage. However, the exact number of molts in the post-embryonic development of horseshoe crabs is not known, and it is not clear how many years they take to mature and how long mature animals live.

According to Shuster, *Limulus polyphemus* takes 9 to 11 years (Shuster, 1960) and 19 or fewer molts (Shuster, 1954) to reach maturity. Based on field observation and measurement of exuvial sizes, Asano (1942) reported that *T. tridentatus* reached maturity in 15 to 16 years after 17 or 18 molts. Goto and Hattori (1929) suggested that *T. tridentatus* molts 12 to 13 times before maturity.

The best way to confirm these animals' growth history would be to follow individual horseshoe crabs from hatching to maturity in their natural habitat. However, this is difficult to do for ten or more years without interruption. The alternative is to rear hatched horseshoe crab juveniles to maturity in a biological laboratory. Thus, Sekiguchi began to rear *T. tridentatus* and *L. polyphemus* from artificially fertilized eggs. The number of first instars used at the starting point was 100 or more for both species. Unfortunately, the last individual of *T. tridentatus* died at the tenth-instar stage, and the last *L. polyphemus* died at the fourteenth-instar stage. The plan of rearing them to adulthood was therefore abandoned.

During the experiment, however, the animals left us many exuviae and body specimens, enabling us to compile data on growth stages.

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Materials and Methods

Japanese horseshoe crabs (*Tachypleus tridentatus*) were collected from Hakata Bay, Fukuoka, Japan. Southeast Asian horseshoe crabs (*Tachypleus gigas* and *Carcinoscorpius rotundicauda*) were collected in the vicinity of Bangsaen, Thailand. American horseshoe crabs (*Limulus polyphemus*) were supplied by the Marine Biological Laboratory, Department of Marine Resources, Woods Hole, Massachusetts.

Just after hatching, *Limulus* individuals were reared in glass bowls (~6 cm diameter, ~3 cm depth) containing seawater of 20, 25, 30, or 35‰ salinity at 30–35°C. Seawater was recycled daily during Sekiguchi's stay in North Carolina (at the Duke University Marine Laboratory). Each glass bowl held 20 individuals. In Japan, *Limulus* juveniles were reared individually after the fifth-instar stage in normal seawater (34–35‰ salinity) at room temperature.

The first-posthatched juveniles of the Asian horseshoe crabs were cultured in bowls (~8.5 cm diameter, ~4.5 cm depth) which held 20 individuals and contained normal seawater. Asian horseshoe crab juveniles were reared individually after the first-instar stage in normal seawater at room temperature.

Individual horseshoe crab juveniles were cultured in seawater containers adequately sized to hold animals at different growth stages. The smallest container was an individual compartment about 34 × 34 × 30 mm deep for *T. tridentatus* second instar (prosomal width ~8 mm, total length ~10 mm), and the largest one was about 36 × 25.5 × 10 cm deep for *Limulus* fourteenth instar (prosomal width ~8 cm, total length ~14 cm). A layer of sand was placed in each compartment and container to allow burrowing. Culture seawater was prepared by filtering normal seawater and was changed daily (re-filtered seawater was sometimes used). Seawater was not recirculated or aerated.

After the second-instar stage, juveniles were fed daily. For each feed they were placed in seawater containers with freshly hatched brine shrimps, chopped earthworms (Tubificidae), or chopped Japanese littlenecks (Veneridae) for about 60–90 minutes.

Body sizes were measured at eight parts of the first instars and at nine parts of animals at growth stages from the second instar to adult, as shown in Figure 1. The sizes of the first to third instars were measured with a micrometer under a stereomicroscope, while the sizes of animals older than the third-instar stage were measured with slide calipers.

Subadult horseshoe crabs are externally similar regardless of sex, except for the gonopores; but after the last molt, the males of the four species are distinguished from the females by the claspers of the second prosomal appendages. The females of *Tachypleus* are distinguishable from males by the three shortened marginal (or movable)

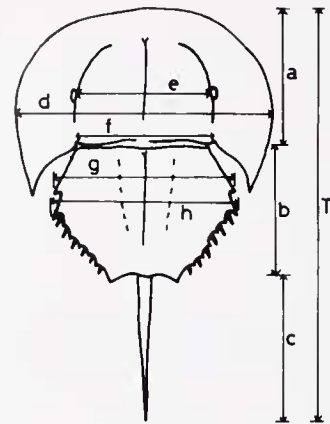


Figure 1. Horseshoe crab measurements. The parts whose lengths are measured are *a-h* and *T*. The first instar has no telson (*c*).

spines (Sekiguchi and Nakamura, 1979). Using these characteristics we determined whether an animal had grown up or not. However, most females of *Limulus* and *Carcinoscorpius* were also considered to be adult animals by their production of mature eggs (which we used for developmental experiments).

Results

Early stages of *Limulus* juvenile growth

The first-instar stage. The lengths of the first-instar stage were 11–16 days at 20‰, 12–20 days at 25‰, 11–20 days at 30‰, and 13–24 days or more at 35‰ when 20 individuals were reared in a glass bowl. Their average values were 13.4, 15.3, 14.6, and 18.6 + α days (the last number, 18.6 + α , means that 17 individuals molted for the first time during the 24 days after hatching), respectively.

The second-instar stage. The second-instar stage lasted 10–12 (11.2) days at 20‰, 10–11 (10.3) days at 25‰, and 10–12 (10.9) days at 30‰ when the lengths were determined using the first 10 individuals grown to the third-instar stage (the average values are shown in parentheses). The growth of juveniles slowed greatly at 35‰.

The third-instar stage. The 12 third-instar juveniles molted for the third time 10–18 days after the second molt in seawater of 30‰ salinity at 30–35°C. The average length of this stage was 12.5 days.

The fourth-instar stage. Eleven of the above 12 fourth-instar juveniles became fifth-instar juveniles 13–20 days after the third molt; the average length of this stage was 15.8 days.

The fifth-instar stage. One of the two fifth-instar juveniles reached the sixth-instar stage 22 days after the

fourth molt; the other reached it 23 days after the fourth molt.

After that, *L. polyphemus* juveniles were cultured in normal seawater (34–35‰) at room temperature. Many fifth-instar juveniles molted to become sixth-instar juveniles between September and mid-November, but no juveniles became seventh-instar juveniles within the first year.

The sixth-instar and later stages. The 23 sixth-instar juveniles of *Limulus* entered a second year, and 17 of them molted 3 times in the second year. The first sixth-molt in our experiment occurred in late April.

During the third year, *Limulus* juveniles molted twice, and the eleventh-instar juveniles entered the fourth year. They molted only once a year from the fourth year to the sixth year. This growth pattern was noted for *Limulus* juveniles whose posthatch development was successful. There were many exceptions: (1) the fifth-instar juveniles which molted four times in the first year molted four times in the second year. (2) Many normal sixth-instar juveniles molted four times in the second year and only once in the third year. (3) Many juveniles molted twice in the fourth year when they had molted only once in the third year.

Several *Limulus* juveniles were grown to the fourteenth-instar stage in the sixth year. They lived for another year or two without molting and then died.

Early stages of *T. tridentatus* juvenile growth

Freshly hatched juveniles of *T. tridentatus* never molted in the first year at room temperature. When they were reared at 30°C year-round, they molted only once late in the first year.

T. tridentatus juveniles molted for the first time in late June, for the second in mid-July, and for the third in mid-September of the second year. The fourth-instar juveniles passed the winter. In the third year, they molted for the fourth time between early June and early July and for the fifth time between early August and late September. Most of them molted once a year from the fourth year to the seventh year. The molts occurred between early June and late July in about 80% of animals in the fourth and fifth year.

Body sizes and relative growth

The mean values and standard deviations of body sizes of *L. polyphemus* from the first to fourteenth instars and of *T. tridentatus* from the first to the tenth instars are shown in Tables I and II, respectively.

The two relative growth patterns are presented by the allometric growth curves in Figure 2 using a logarithmic plot. (Their correlation coefficients were near 1.) The lin-

Table I
The means \pm SD (standard deviations) of body sizes (mm) of *Limulus polyphemus* juveniles continuously reared from the 1st-instar to 14th-instar stages

	Instar stage													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
a	2.14 \pm 0.30	3.06 \pm 0.34	4.46 \pm 0.24	5.70 \pm 0.44	7.08 \pm 0.57	8.80 \pm 0.63	11.2 \pm 0.83	13.9 \pm 1.10	17.6 \pm 1.79	23.0 \pm 2.00	26.5 \pm 3.82	31.0 \pm 3.23	38.7 \pm 5.34	48.0 \pm 3.03
b	1.91 \pm 0.25	2.12 \pm 0.17	3.11 \pm 0.21	4.06 \pm 0.33	5.11 \pm 0.46	6.41 \pm 0.50	8.18 \pm 0.59	10.4 \pm 0.81	13.2 \pm 1.20	16.8 \pm 1.37	18.9 \pm 2.16	22.5 \pm 2.61	28.6 \pm 3.86	35.6 \pm 2.57
c		1.73 \pm 0.19	3.44 \pm 0.30	5.07 \pm 0.60	7.04 \pm 0.74	9.41 \pm 0.96	12.8 \pm 1.22	17.1 \pm 1.66	23.0 \pm 2.06	30.4 \pm 3.65	35.5 \pm 4.19	43.7 \pm 6.84	59.7 \pm 8.83	68.0 \pm 3.00
d	3.30 \pm 0.30	5.03 \pm 0.20	7.03 \pm 0.41	9.08 \pm 0.87	11.3 \pm 1.05	14.5 \pm 1.18	18.6 \pm 1.39	23.5 \pm 1.78	29.5 \pm 2.31	38.0 \pm 2.93	43.7 \pm 4.95	50.2 \pm 7.30	64.4 \pm 8.87	81.2 \pm 5.27
e		2.90 \pm 0.22	4.00 \pm 0.20	5.03 \pm 0.35	6.19 \pm 0.46	7.60 \pm 0.55	9.69 \pm 0.75	12.2 \pm 0.84	15.6 \pm 1.30	19.8 \pm 1.59	23.9 \pm 2.03	28.3 \pm 3.94	35.3 \pm 4.20	44.0 \pm 3.34
f	2.04 \pm 0.20	2.98 \pm 0.18	4.29 \pm 0.27	5.41 \pm 0.42	6.75 \pm 0.57	8.46 \pm 0.62	10.7 \pm 0.79	13.6 \pm 1.00	17.3 \pm 1.43	21.6 \pm 2.22	25.2 \pm 2.17	29.5 \pm 3.52	37.0 \pm 4.20	45.8 \pm 4.00
g		3.45 \pm 0.18	4.83 \pm 0.31	6.12 \pm 0.54	7.62 \pm 0.76	9.53 \pm 0.77	12.1 \pm 0.93	15.2 \pm 1.16	19.0 \pm 1.53	23.6 \pm 1.70	27.4 \pm 2.87	31.5 \pm 3.99	39.8 \pm 4.71	48.2 \pm 4.35
h	2.87 \pm 0.62	3.56 \pm 0.20	4.91 \pm 0.25	6.19 \pm 0.49	7.73 \pm 0.60	9.67 \pm 0.65	12.3 \pm 0.83	15.5 \pm 1.16	19.3 \pm 1.57	25.2 \pm 2.40	27.7 \pm 3.11	33.2 \pm 3.90	41.7 \pm 5.60	51.4 \pm 4.27
T	4.05 \pm 0.48	6.89 \pm 0.59	11.0 \pm 0.68	14.8 \pm 1.21	19.1 \pm 1.43	24.6 \pm 1.74	32.1 \pm 2.43	41.1 \pm 3.25	54.3 \pm 5.03	70.9 \pm 6.46	80.0 \pm 9.41	97.9 \pm 10.9	127.8 \pm 15.2	141.0
n	44	36	37	47	76	74	73	65	53	24	19	17	9	1

Body parts (*a*–*h* and *T*) measured are shown in Fig. 1.

The *n* shown in the lowest row represents a sample size for part *T*. It is the smallest in the sample sizes for parts *a* to *T* at each growth stage mainly because of imperfect telson exuviae (for example, sample sizes for *a* to *T* at stage 14 are 5, 5, 2, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 1, respectively).

Table II

The means ± 1 SD (standard deviations) of body sizes (mm) of *Tachypleus tridentatus* juveniles continuously reared from the 1st-instar to 10th-instar stages

	Instar stage									
	1	2	3	4	5	6	7	8	9	10
<i>a</i>	3.32 \pm 0.18	4.30 \pm 0.41	5.76 \pm 0.49	7.83 \pm 0.31	10.2 \pm 0.40	12.3 \pm 0.58	15.7 \pm 1.02	19.8 \pm 1.24	23.6 \pm 0.75	25.0
<i>b</i>	3.03 \pm 0.17	3.06 \pm 0.18	4.25 \pm 0.17	5.58 \pm 0.18	7.37 \pm 0.25	9.18 \pm 0.95	12.2 \pm 0.94	15.8 \pm 0.81	18.2 \pm 0.33	21.1
<i>c</i>		2.60 \pm 0.26	6.13 \pm 0.46	9.93 \pm 0.54	14.9 \pm 0.92	21.1 \pm 1.64	27.1 \pm 2.73	38.0 \pm 3.29	46.6 \pm 0.70	53.1
<i>d</i>	5.98 \pm 0.31	7.75 \pm 0.36	10.7 \pm 0.45	13.3 \pm 0.53	17.5 \pm 0.80	22.2 \pm 1.44	28.0 \pm 2.14	37.3 \pm 2.92	42.7 \pm 0.51	48.4
<i>e</i>	3.80 \pm 0.14	4.49 \pm 0.23	6.10 \pm 0.26	7.82 \pm 0.33	9.88 \pm 0.48	12.6 \pm 0.51	16.0 \pm 1.05	20.3 \pm 0.97	23.4 \pm 0.91	26.6
<i>f</i>	3.89 \pm 0.12	4.50 \pm 0.35	6.20 \pm 0.24	8.05 \pm 0.34	10.3 \pm 0.51	12.8 \pm 0.62	16.2 \pm 0.84	20.2 \pm 1.07	23.6 \pm 1.11	26.1
<i>g</i>	4.85 \pm 0.17	6.00 \pm 0.15	7.86 \pm 0.28	9.96 \pm 0.44	12.8 \pm 0.61	16.1 \pm 0.86	19.9 \pm 1.22	26.1 \pm 1.66	28.8 \pm 0.90	32.1
<i>h</i>	4.82 \pm 0.13	5.77 \pm 0.23	7.78 \pm 0.27	10.0 \pm 0.36	13.0 \pm 0.60	16.3 \pm 0.92	20.6 \pm 1.25	26.8 \pm 1.73	30.0 \pm 1.69	34.7
<i>T</i>	6.30 \pm 0.19	9.95 \pm 0.71	16.4 \pm 0.77	23.3 \pm 0.91	32.3 \pm 1.19	42.6 \pm 2.49	54.9 \pm 4.25	74.4 \pm 3.65	88.7 \pm 0.40	99.2
<i>n</i>	33	22	12	13	13	21	24	9	2	1 ¹

¹ All the sample sizes for *a* to *T* are 1.

Body parts (*a*–*h* and *T*) measured are shown in Fig. 1.

The sample size for *T* at each growth stage are shown in the lowest row (*n*). Generally it is smallest in the sample sizes for parts *a* to *T* (for example, sample sizes for *a* to *T* at stage 9 are 3, 3, 2, 3, 3, 3, 3, 3, and 2, respectively).

ear growth correlation means that the two parts are grown in proportion to each other, and a change in the slope means that a difference in growth rate between the two parts occurred. The growth curves other than that of the telson show only a straight line, suggesting that the growth correlation between prosoma width and the length of each of seven parts (except the telson) was invariable through post-embryonic development of *Limulus*. (Their relative growth coefficients are about 1 except for that of the total length.) On the other hand, the growth correlation between prosomal width and telson length changed twice: the curve showed the three phases. The first was the second through fourth instar, the second was the fourth through twelfth instar, and the third was the twelfth instar through adulthood (Fig. 2). The relative growth coefficients were 1.83, 1.23, and 1.03 for the first, second, and third phases, respectively.

In *T. tridentatus*, the allometric growth curves in a logarithmic plot showed a straight line except those for telson length and total length. The growth curves for the telson and total length consisted of three and two straight lines, respectively.

These states of growth were also characteristic of *T. gigas* and *C. rotundicauda*, although the body sizes of only five stages, from the first to fourth instars and the adult, were available for *T. gigas* and *C. rotundicauda*.

The estimation of growth stages

Stepwise growth after the fourteenth instar of *L. polyphemus* and the tenth instar of *T. tridentatus* could not

be observed. However, it could be estimated using growth data from the first instar to the fourteenth instar (in *L. polyphemus*) or to the tenth instar (in *T. tridentatus*).

The rate of increase in the stepwise growth of each part is the most important information for estimating unknown animal sizes after the tenth-instar stage (*T. tridentatus*) and the fourteenth-instar stage (*L. polyphemus*). The rate of increase was 1.25 (*g* and *h*) to 1.37 (*c*) in *L. polyphemus*, and the average value was 1.28 for the nine parts (Table III). As shown in Figure 2, the points indicating adult and juvenile sizes were placed on an allomet-

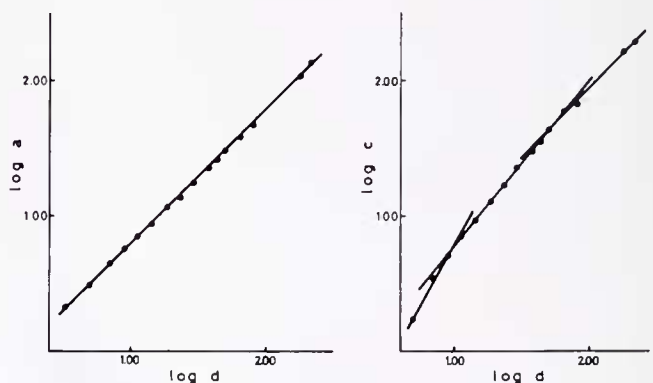


Figure 2. Relative growth in *Limulus polyphemus*. The allometric growth curve displays the logarithmic correlation between prosomal width (*d*) and prosomal length (*a*) or telson length (*c*). The uppermost and second uppermost dots on the lines present the mean sizes of females and males, respectively.

Table III

The presumed sizes (mm) of *Limulus polyphemus* juveniles after the 14th-instar stage and means ± 1 SD (standard deviations) of adult sizes (mm) of *L. polyphemus*

	Rate of increase	Instar stage					Adult	
		15	16	17	18	19	♂	♀
a	1.27	61	77	98	125	201	109.7 \pm 7.0	138.5 \pm 19.2
b	1.26	45	57	71	90	113	85.3 \pm 4.6	106.3 \pm 13.2
c	1.37	—	—	—	—	—	165.0 \pm 20.2	194.5 \pm 33.1
d	1.28	104	133	170	218	279	177.4 \pm 11.4	213.9 \pm 30.8
e	1.26	55	70	88	111	140	105.2 \pm 7.5	133.6 \pm 18.6
f	1.27	58	74	94	119	151	104.0 \pm 6.9	130.0 \pm 17.4
g	1.25	60	75	94	118	147	103.1 \pm 6.5	129.1 \pm 16.2
h	1.25	64	80	100	125	157	117.6 \pm 7.3	148.8 \pm 19.3
T	1.32	186	246	324	428	565	356.5 \pm 31.6	437.9 \pm 54.7

a-h and T are shown in Fig. 1.

The presumed sizes were calculated using the mean sizes of the 14th instars.

—, The size was not presumed.

ric growth line. Based on these results, the sizes of the respective parts after the fourteenth-instar stage were calculated using the appropriate rates of increase and mean sizes of the fourteenth instars (Table III). Similarly, the sizes after the thirteenth-instar stages of *T. tridentatus* were estimated and are shown with adult sizes in Table IV.

The presumptive numbers of growth (instar) stages of adult animals are summarized in Table V. From these data, we conclude that *Limulus polyphemus* males generally molt 16 times to reach maturity, and females, 17 times. Similarly, it is concluded from the presumptive

numbers of growth stages (Table V) that *T. tridentatus* males generally molt 15 times to reach maturity, and females, 16 times.

We have few data on the growth stages of *T. gigas* and *C. rotundicauda*. However, it is thought that the stepwise growth of these two species progresses at almost the same rate as that of *L. polyphemus* or *T. tridentatus*, and thus their unknown body sizes after the fourth-instar stage were calculated (Tables VI, VII). According to these growth tables, *T. gigas* males molt 12 times to reach adulthood, and females, 13 times. *C. rotundicauda* reaches maturity after the thirteenth molt in both males

Table IV

The presumed sizes (mm) of *Tachypleus tridentatus* juveniles after the 13th-instar stage and means ± 1 SD (standard deviations) of adult sizes (mm) of *T. tridentatus*

	Rate of increase ¹	Instar stage					Adult	
		14	15	16	17	18	♂	♀
a	1.28	81	104	133	170	218	133.2 \pm 12.6	166.1 \pm 12.0
b	1.26	58	73	92	116	146	114.4 \pm 12.9	136.6 \pm 6.7
c	1.54	—	—	—	—	—	260.5 \pm 31.7	298.8 \pm 32.4
d	1.28	147	188	240	308	394	244.2 \pm 18.7	278.4 \pm 16.7
e	1.26	74	94	118	149	187	132.9 \pm 7.0	164.4 \pm 10.5
f	1.25	72	90	113	141	176	129.9 \pm 8.1	157.6 \pm 9.6
g	1.25	88	110	137	172	215	148.3 \pm 10.2	177.4 \pm 11.3
h	1.26	95	120	151	191	240	160.1 \pm 11.1	188.5 \pm 10.6
T	1.40	477	668	935	—	—	509.0 \pm 49.9	600.4 \pm 33.4

¹ Each mean rate of increase was determined using growth data from the 1st-instar to 9th-instar stages because of only one small 10th instar.

a-h and T are shown in Fig. 1.

The presumed sizes were calculated using the mean sizes of the 9th instars.

—, The size was not presumed.

Table V

The presumptive numbers of the growth (instar) stage of adults of *Limulus polyphemus* and *Tachypleus tridentatus*

		a	b	d	e	f	g	h
<i>L. polyphemus</i>	♀	18	19	18	19	18	18	19
	♂	17 (~18)	18	17	18	17 (~18)	17 (~18)	(17~) 18
<i>T. tridentatus</i>	♀	17	18	(16~) 17	17 ~ 18	17 ~ 18	17	17
	♂	16	17	16	16 ~ 17	(16~) 17	16	16

a, b, d, e, f, g, and h are shown in Fig. 1.

and females. The rate of increase (1.26) used in the calculation was a mean rate of increase in *Limulus* body sizes except for the telson and total length.

Discussion

As the basis for estimating the total number of molts, it is necessary to know the relative growth of each part in *L. polyphemus* and *T. tridentatus*. Because the width of the prosoma (d) is easily measured and yields the smallest error in measurement and because Waterman (1954) and Shuster (1955) studied the relative growth of horseshoe crabs using the width of the prosoma, the relative growth between the width of the prosoma and each of eight lengths (a, b, c, e, f, g, h, and T) was examined. The growth of the *Limulus* telson was fastest in the first phase, and it preserved positive allometry in the second phase. Finally, the growth rate of the telson became similar to the widening of the prosoma. Because this tendency of telson growth was

detected in *T. tridentatus*, the fact that the telson growth curve shows the three phases seems to be a remarkable characteristic of horseshoe crab growth in all species. However, positive allometry for the telson is predictable since the adult has a long telson but the first instar has none.

Waterman (1954) also indicated that the number of ommatidia in the horseshoe crab compound eye showed markedly rapid development in the initial growth phase and that telson elongation was marked by positive allometry during initial growth. The relative growth coefficients of six parts (not including the length of the telson and total length) are about 1 in both *L. polyphemus* and *T. tridentatus*. This means that each part except the telson enlarges at the same rate as does the width of the prosoma. Therefore, with the exception of the telsons, the shapes of the juveniles (except the first instar) and of mature adults are similar. Thus, we could estimate the juveniles' sizes after the tenth-instar (*T. tridentatus*) and

Table VI

The measured (stages 1 to 4) and presumed (stages 12 to 15) sizes (mm) of *Tachypleus gigas* juveniles and means \pm 1 SD (standard deviations) of *T. gigas* adult sizes (mm)

	Instar stage				Rate of increase	Instar stage				Adult	
	1	2	3	4		12	13	14	15	♂	♀
a	4.08	6.26	8.19	10.1	1.26	64	81	102	128	86.8 \pm 4.9	116.0 \pm 8.8
b	3.19	4.90	6.25	8.00	1.26	51	64	81	102	74.1 \pm 3.1	99.1 \pm 5.6
c	—	3.92	7.81	12.9	—	—	—	—	—	171.0 \pm 15.2	206.2 \pm 19.5
d	8.33	12.1	14.8	19.2	1.26	122	154	194	244	163.0 \pm 7.4	210.0 \pm 15.6
e	4.51	5.57	7.01	9.27	1.26	59	74	93	118	80.1 \pm 3.3	110.0 \pm 6.4
f	4.53	6.04	7.66	9.73	1.26	62	78	98	124	82.4 \pm 3.4	113.2 \pm 14.8
g	5.63	7.94	9.80	12.2	1.26	78	98	123	155	89.9 \pm 3.3	120.0 \pm 6.9
h	6.23	9.20	10.8	13.5	1.26	86	108	136	172	107.5 \pm 13.0	135.6 \pm 7.4
T	—	—	—	—	—	—	—	—	—	331.6 \pm 20.0	422.2 \pm 29.1
n	10	10	10	7	—	—	—	—	—	23	30

a-h and T are shown in Fig. 1.

The sample sizes are shown in the lowest row (n).

The presumed sizes were calculated using the mean sizes of the 4th instars.

—, The size was not measured or presumed.

Table VII

The measured (stages 1 to 4) and presumed (stages 13 to 15) sizes (mm) of *Carcinoscorpius rotundicauda* juveniles and means \pm 1 SD (standard deviations) of *C. rotundicauda* adult sizes (mm)

	Instar stage				Rate of increase	Instar stage			Adult	
	1	2	3	4		13	14	15	♂	♀
<i>a</i>	2.56	3.60	4.96	6.95	1.26	56	70	88	68.8 \pm 3.6	74.1 \pm 6.2
<i>b</i>	2.09	2.87	4.22	5.63	1.26	45	57	72	59.3 \pm 3.0	62.4 \pm 4.7
<i>c</i>	—	1.55	4.08	7.56	—	—	—	—	154.1 \pm 9.1	160.6 \pm 20.2
<i>d</i>	5.02	7.57	10.2	12.4	1.26	99	125	158	128.7 \pm 3.7	133.8 \pm 8.2
<i>e</i>	2.73	3.43	4.43	6.01	1.26	48	61	76	58.7 \pm 2.2	63.7 \pm 4.4
<i>f</i>	2.78	3.86	4.82	6.51	1.26	52	66	83	64.0 \pm 2.5	68.5 \pm 4.4
<i>g</i>	3.50	4.86	6.74	8.55	1.26	68	86	109	87.0 \pm 2.6	89.8 \pm 7.6
<i>h</i>	3.70	5.11	7.03	9.05	1.26	72	91	115	96.0 \pm 3.3	99.6 \pm 5.8
<i>T</i>	—	—	—	—	—	—	—	—	283.7 \pm 11.0	296.6 \pm 30.9
<i>n</i>	10	10	10	10	—	—	—	—	22	23

a-h and *T* are shown in Fig. 1.

The sample sizes are shown in the lowest row (*n*).

The presumed sizes were calculated using the mean sizes of the 4th instars.

—, The size was not measured or presumed.

fourteenth-instar (*L. polyphemus*) stages using the rate of increase in the stepwise growth of each part (Tables III, IV).

According to the growth table, we could determine the growth stages of two *Limulus* juveniles sent from Woods Hole. One individual estimated to be at the fourteenth-instar stage molted once and died. The rate of increase in this animal was 1.28 on average. Another individual was estimated to be at the seventeenth-instar stage. This animal also molted once; the rate of increase was 1.16. These facts strongly indicate that the rate of increase obtained from juveniles in the culture experiments is almost the same as that from the animals in their natural habitat.

However, there were some small mature males among the *L. polyphemus* specimens sent from Woods Hole in 1982. The total length of the smallest one was 229 mm and its prosomal width was 114 mm, suggesting that the sizes of these small males were similar to those of the presumptive sixteenth-instar juveniles. We cannot say whether they became adults after the fifteenth molt or became small-sized adults after the sixteenth molt because of the small rate of increase. According to Shuster (1982), in a local population (Plum Island Sound, Massachusetts) composed of a small type of *Limulus*, the mean prosomal width for males was 118 mm, and for females, 158 mm. It seems that *Limulus* in this population probably molted one fewer times than other *Limulus* to reach maturity—that is, 15 times in males and 16 times in females (Table III). On the other hand, the largest adults were found in the population at Bird Shoal,

North Carolina, with a mean prosomal width of 232 mm for males and 327 mm for females. According to the growth table (Table III), these largest *Limulus* seem to have molted 17 times in males and 19 times in females. Thus, it is suggested that some *Limulus* reach maturity after one fewer or one more molt than normal animals, which molt 16 times in males and 17 times in females.

As in *Limulus*, the largest and the smallest *T. tridentatus* seemed to molt one more and one fewer times to reach maturity than normal animals in both females and males. When these results are compared with those of Goto and Hattori (1929), there are some obvious discrepancies between the two during stepwise growth. Although Goto and Hattori (1929) measured the body size of 1477 individuals (including 181 exuviae) of *T. tridentatus* from the first-instar stage to adulthood, their results were quite different from ours. However, they were far-sighted in suggesting that females reached maturity after one more molt than males.

When we estimated the unknown body sizes of juveniles in *T. gigas* and *C. rotundicauda*, the growth rate used, 1.26, was a mean rate of increase in *Limulus* body sizes except for the telson and total length. This value is coincident with the mean values (1.26) calculated using the prosoma lengths (*a*) and opisthosoma widths (*h*) of five sets of Chinese *T. tridentatus* before and after a given molt (Chou and Cheng, 1950). According to the growth table (Table IV), *T. tridentatus* of the five sets molted for the fifth time (two sets), sixth time, seventh time, and eighth time. Chou and Cheng (1950) reported that the

rates of increase in four other parts were 1.32 (*b*), 1.3 (*c*), 1.21 (*d*), and 1.21 (*T*).

Based on our estimation of the growth stages of horseshoe crab juveniles, there is a one-step difference in the instar stage between males and females of *L. polyphemus*, *T. tridentatus*, and *T. gigas*. There is no difference in the instar stage between males and females of *C. rotundicauda*. If the discrepancy is real, it is very interesting.

The horseshoe crabs lay their eggs in the sand near the high-tide level during the spring tide and there the eggs develop prior to hatching. The salinity of seawater was 18–33‰ on the Tataru coast, Saga, Japan, where *T. tridentatus* lays eggs (Sugita *et al.*, 1985). In Barnstable Harbor, on the north shore of Cape Cod, Massachusetts, where nests of *Limulus* eggs were found, the salinity was 20–32‰ (Robertson, 1970). Therefore, horseshoe crab embryos and hatched larvae and juveniles were expected to be tolerant of a wide range of environmental salinity levels. When the embryos were reared in seawater of 20–30‰, the developmental time until hatching was almost the same (Jegla and Costlow, 1982; Sugita *et al.*, 1985). When the post-hatch larvae and juveniles were reared in seawater of 20–30‰, it seemed that the difference in the effect of salinity on their growth was not significant (Jegla and Costlow, 1982; Laughlin, 1983). However, the development and growth of horseshoe crabs were slightly delayed in seawater with 35‰ salinity.

In our rearing experiment, the significant effect of salinity on the growth of *Limulus* young juveniles was not detected in 20–30‰. However, growth was delayed in 35‰. Regardless, this delay in juvenile growth did not affect the total number of molts in the first year.

From our rearing experiment, it is clear that *L. polyphemus* juveniles reach the sixth-instar stage within a year of when they were laid. Jegla and Costlow (1982) reported that freshly hatched juveniles of *L. polyphemus* molted about six times during the first year and several additional times during the second. However, no *Limulus* juveniles molted six times in our culture experiment during the first year. If the sixth instars had molted one more time during the first year in our rearing experiment, the sixth molt would have taken place in November or December. According to our records of the progress of *Limulus* growth, however, the number of *Limulus* juveniles that molt during this season is extremely small, probably due to the decline in temperature. Thus, it is thought to be unusual for *Limulus* juveniles to molt six times in the first year unless they are reared in a thermostatically controlled room. If the *Limulus* juveniles had been reared continuously in North Carolina, they might have molted for the sixth time.

Barlow *et al.* (1982, 1986) reported that light and vision play an important role in *Limulus* mating and

breeding behavior. For juvenile growth, however, it seemed that the influence of light was not serious, because there was no difference in substantial growth of *Limulus* juveniles between the culture experiment, in which juveniles were treated with a 13/11 light/dark regimen (Jegla and Costlow, 1982), and our culture experiment, in which the lighting conditions depended on room conditions.

T. tridentatus juveniles up to tenth-instar stage (presumed according to our growth table) dwell at the tideland and, when the land is uncovered by the diurnal fall of the tide, they emerge from muddy sand and take food in tide pools (Kawahara, 1984). Therefore, it appears that the frequency of feeding in our rearing experiment was fairly adequate to grow horseshoe crab juveniles.

When the post-embryonic development of *L. polyphemus* goes smoothly, the animals molt five times in the first year, three times in the second year, twice in the third year, and once in the fourth year, after which they become twelfth-instar juveniles. It seems that after the twelfth instar they molt once a year, although there is no strong evidence for this assumption. On the other hand, the presumed adult stages of *L. polyphemus* correspond to the seventeenth-instar stage for males and to the eighteenth-instar stage for females. Therefore, *L. polyphemus* males reach maturity in the ninth year and females in the tenth year, providing that they molt once a year after the fourth year.

Shuster (1982) reported that the molting and aging sequence in Atlantic coast populations of *Limulus* generally included stage I–V in the first year, VI and VII in the second year, VIII and IX in the third year, and then a single molt each year from then on, with males reaching maturity at stage XVI in the ninth year; females had at least one more stage (XVII), reaching maturity one year later than males. However, Shuster determined the growth stages and ages using eight exuviae derived from a female *Limulus* and many immature and adult animals collected in the field, so we cannot compare our data precisely with his data, although his conclusion is fairly close to ours.

In *T. tridentatus*, the first instars pass the winter and molt three times in the second year, twice in the third year, and once in the fourth year, reaching the seventh-instar stage. After this they molt once a year. The sizes of male and female adult *T. tridentatus* corresponded to those of the presumptive sixteenth- and seventeenth-instar juveniles, respectively. Thus, male *T. tridentatus* are mature in the thirteenth year while females are mature in the fourteenth year.

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